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Discovery and characterization of a molecular marker for *Sclerotinia minor* (Jagger) resistance in peanut

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Abstract The production of cultivated peanut, an important agronomic crop throughout the United States and the world, is consistently threatened by various diseases and pests. *Sclerotinia minor* Jagger (*S. minor*), the causal agent of Sclerotinia blight, is a major threat to peanut production in the Southwestern US, Virginia and North Carolina. Although information on the variability of morphological traits associated with Sclerotinia blight resistance is plentiful, no molecular markers associated with resistance have been reported. The identification of markers would greatly assist peanut geneticists in selecting genotypes to be used in breeding programs. The main objective of this work was to use simple sequence repeat (SSR) primers previously reported for peanut to identify a molecular marker associated with resistance to *S. minor*. Out of 16 primer pairs used to examine peanut genomic DNA from 39 different genotypes, one pair produced bands at approximately 145 and 100 bp, consistent with either *S. minor* resistance or susceptibility, respectively. Cloning and sequencing of these bands revealed the region is well conserved among all genotypes tested with the exception of the length of the SSR region, which varies with disease resistance levels. This is the first report of a molecular marker associated with resistance to Sclerotinia blight in peanut. The identification of this marker and development of a PCR-based screening method will prove to be extremely useful to peanut breeders in screening germplasm collections and segregating populations as well as in pyramiding *S. minor* resistance with other desirable traits into superior peanut lines.

Keywords Molecular marker · Peanut · *Arachis hypogaea* L. · Sclerotinia blight · Disease resistance

Abbreviations

ABL	Advanced breeding line
AFLP	Amplified fragment length polymorphism
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SCAR	Sequence characterized amplified region
SSR	Simple sequence repeat

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Cultivated peanut (*Arachis hypogaea* L.) is a self-pollinated allotetraploid ($2n = 4x = 40$), which is

economically important throughout the world (Kochert et al. 1991). Peanut is susceptible to many pathogens, with most damage being caused by fungi (Melouk and Backman 1995). Soil-borne fungi cause diseases that adversely affect peanut health and productivity throughout the growing areas of the United States. Diseases such as pod rot (*Rhizoctonia solani* Kühn, *Pythium myriotylum*), crown rot (*Aspergillus niger* Teigh) and southern blight (*Sclerotium rolfsii* Sacc) occur in all US peanut-producing areas, while others such as Sclerotinia blight (*Sclerotinia minor* Jagger) are limited to certain geographic regions. Sclerotinia blight is of major concern to peanut producers in the Southwest US. Early symptoms of Sclerotinia blight include wilting and stem lesions with white mycelium growth. Progression of the disease can be rapid under optimal environmental conditions, which include a cool and damp plant canopy, ultimately resulting in light tan lesions on stems, stem shredding, and plant death. Depending upon severity of field infestation, yield losses due to Sclerotinia blight are typically 10% but may be as high as 50% (Melouk and Backman 1995). Expensive fungicide applications throughout the growing season are often required for effective disease management. Recent reductions in the US peanut price support have increased the urgent need for a less expensive and more effective means of disease control.

Host plant resistance would provide the most effective solution to managing Sclerotinia blight. Traditional breeding and screening practices have resulted in cultivars with partial resistance that are suitable for production in the southwest (Smith et al. 1991, 1998; Baring et al. 2006), but most resistant cultivars released prior to 2006 did not contain the high oleic acid trait which is highly desired by the peanut industry. Several factors contribute to the lack of available Sclerotinia blight resistant cultivars. The mechanism of host resistance is not well understood. Plant morphology can play an important role in resistance to fungal disease because of the environment required for development and progression (Chappell et al. 1995; Coffelt and Porter 1982; Coyne et al. 1974; Schwartz et al. 1978). Plant types with a more upright growth habit and open canopy, such as Spanish, appear to be more resistant than those with a dense canopy (such as runner and Virginia types) which allows for temperature reduction and moisture accumulation. However, the mechanism of resistance among Spanish types is not purely morphological since the Spanish cultivars Pronto and Spanco are as susceptible as many runner types, suggesting contribution by a genetic component. Inheritance of the resistance trait has not been well studied but Wildman et al. (1992) suggested that at least two loci were involved in Sclerotinia blight resistance among genotypes studied. Cytoplasmic factors have also been suggested to be involved in Sclerotinia blight resistance (Coffelt and Porter 1982).

Due to the quantitative nature of resistance, breeding for Sclerotinia blight resistance has relied heavily on traditional field screening methods (Akem et al. 1992; Chappell et al. 1995; Goldman et al. 1995), which can take several years to generate consistent results, and requires large quantities of genetically uniform material. In an effort to accelerate the screening process, Melouk et al. (1992) developed a technique for testing detached shoots of peanut plants for resistance in the greenhouse, producing results that correlate well with field studies. Although reliable, the greenhouse testing method can be hindered by space, personnel, and availability of uniform genetic material.

With the advent of molecular mapping techniques, including molecular markers associated with quantitative traits, rapid advances have been made in improving the efficiency of breeding programs for cropping systems. As early as the 1980s, quantitative trait loci (QTLs) were being identified and used to improve corn (Stuber and Edwards 1986; Stuber et al. 1987). The development of markers associated with disease resistance quickly followed as well as the development of linkage and chromosomal maps. Partial genetic maps and molecular markers associated with disease resistance are now available for many legumes, family Fabaceae, including but not limited to *Glycine max*, *Medicago truncatula*, *Medicago sativa*, *Phaseolus vulgaris*, *Pisum sativum*, *Cicer arietinum*, *Lens culinaris*, *Lotus japonicus*, and *Trifolium pretense* (Gonzales et al. 2005; Ohmido et al. 2007; Sandal et al. 2002).

Until recently, very little genetic diversity could be found in cultivated peanut using molecular markers. Techniques such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLPs), and amplified fragment length polymorphisms (AFLPs) revealed little variation among peanut cultivars (He and Prakash 1997; Kochert et al. 1991; Stalker and Mozingo 2001). However, using these techniques did enable the identification of

molecular markers associated with resistance to nematodes (Garcia et al. 1996; Burow et al. 1996) and the aphid vector of groundnut rosette virus (Herselman et al. 2004) and the construction of a partial linkage map (Burow et al. 2001; Gonzales et al. 2005). Recently, Chu et al. (2007) converted RFLP markers to sequence characterized amplified region (SCAR) markers so as to develop a PCR-based marker system to screen for nematode resistance in peanut. Hopkins et al. (1999) used simple sequence repeat (SSR) primers to uncover six polymorphic SSRs in cultivated peanut and were able to differentiate 15 of 19 accessions tested. Since that discovery, the number of SSR markers has increased (He et al. 2005). Unfortunately, no other molecular markers associated with disease resistance in peanut, including resistance to Sclerotinia blight, have been reported.

In this study, SSR primers for peanut reported by Ferguson et al. (2004) were used to examine the genetic diversity among 39 peanut genotypes specifically selected for their well demonstrated levels of resistance to Sclerotinia blight. The objectives of this work were to identify an amplicon(s) consistent with either disease resistance or susceptibility, clone, and sequence the identified amplicon(s) and develop a PCR-based system to select for Sclerotinia blight resistance in peanut.

Materials and methods

Plant materials

Table 1 lists the 39 peanut genotypes examined in this study. The genotypes included encompass all four US peanut market-types and consist of released cultivars (CV), advanced breeding lines (ABL), and plant introductions to the USDA-ARS peanut germplasm collection.

Field testing

A multi-year study (1997–2006) was conducted to evaluate peanut lines for resistance to Sclerotinia blight using methods previously described (Akem et al. 1992). All genotypes included in this study are listed in Table 1. Field plots were established as a randomized compete block with four replications at the Caddo Research Station, Ft. Cobb, Oklahoma. Soil was Tremona loamy fine sand and the field site was nearly level to slightly sloping. A plot consisted of two 6 m rows spaced at 0.91 m. Seed treated with TOPS 90 fungicide (Gustafson, Plano, TX), at 2.5 g kg^{-1} seed was planted on 15 May (±5 days) each year at a rate of 18 seeds per m at a depth of 4 cm. Sclerotial density of *S. minor* was 2–3 sclerotia per 100 g of soil. Plots were irrigated as needed to ensure good growth and standard agronomic practices were followed throughout the growing season to manage foliar diseases according to the peanut production guide for Oklahoma (Oklahoma State University Cooperative Extension Service Circular E-806). Incidence of Sclerotinia blight (%) in the plots was read approximately 2–3 weeks prior to digging on 15 October (±7 days, depending upon market type maturity) of each year. An infection locus is defined as an area of blight symptoms equal to or less than 15 cm in a standard row. Percent Sclerotinia blight was calculated by dividing the number of infection loci by the number of potential infection loci and multiplying by 100.

DNA extraction

DNA was extracted from each genotype listed in Table 1, either from dry, mature seed (Chenault and Maas 2005) or from young leaf tissue. In case of the latter, 0.2 g of unfolded leaflet tissue was collected from each plant, de-veined, ground in liquid N_2 to a fine powder and vortexed in 1.5 ml extraction mixture [1:1, extraction buffer (0.1 M Glycine-NaOH, pH 9.0, 50 mM NaCl, 10 mM EDTA, 2% SDS, 1% Na-lauryl sarcosine): phenol–chloroform–isoamyl alcohol (25:24:1)]. Extraction mixtures were shaken vigorously for 10 min and then microfuged for 15 min at 10 K rpm at room temperature. DNA was precipitated from the upper layer of each sample by the addition of 750 µl of isopropanol followed by gentle inversion. DNA was spooled onto a glass hook, washed with 70% ethanol, and allowed to air dry for 15 min at room temperature. Hooks were then placed into tubes containing 1 ml extraction buffer and DNA was re-suspended overnight.

DNA suspensions were then incubated with 50 µg Proteinase K for 30 min at 37°C. Proteins and other remaining cellular debris were removed by extraction with phenol–chloroform–isoamyl alcohol (25:24:1) followed by extraction with half volume of chloroform

Table 1 Complete listing of genotypes included in this study along with their respective market types (MT), presence or absence of marker, percent Sclerotinia blight (SB) and reference data

Genotype	MT	Marker[a]	SB (%)	Origin[b]	Reference/Source
ARSOK-R1	R	B	31 ± 3	ABL	USDA-ARS, Stillwater, OK
ARSOK-R2	R	B	32 ± 3	ABL	USDA-ARS, Stillwater, OK
Flavor Runner 458	R	S	54 ± 5	CV	Horn et al. (2001)
Florunner	R	S	68 ± 3	CV	Knauft and Gorbet (1989)
Georgia Green	R	B	28 ± 3	CV	Branch (1996)
Georgia Hi-O/L	R	B	19 ± 3	CV	Branch (2000)
Grif 13838	S	B	12 ± 2	Ecuador	USDA-ARS germplasm collection
Jupiter	VR	b	39 ± 5	CV	Okla State Univ Ag Exp Station 2000
N96076L	VR	b	12 ± 3	CV	Isleib et al. (2006)
N. Mexico Valencia C	V	B	17 ± 3	CV	Hsi (1980)
N03076FT	VR	b	22 ± 9	ABL	Isleib, NC State University
N03081T	VR	b	22 ± 9	ABL	Isleib, NC State University
N03084FT	VR	S	15 ± 8	ABL	Isleib, NC State University
N03085FT	VR	S	14 ± 4	ABL	Isleib, NC State University
N03086FT	VR	S	10 ± 4	ABL	Isleib, NC State University
N03088FT	VR	S	4 ± 2	ABL	Isleib, NC State University
N03089T	VR	S	12 ± 2	ABL	Isleib, NC State University
N03090T	VR	S	21 ± 12	ABL	Isleib, NC State University
Okrun	R	S	66 ± 3	CV	Banks et al. (1989)
PI 259796	R	L	2 ± 1	Malawi	USDA-ARS germplasm collection
PI 274193	R	L	7 ± 3	Bolivia	USDA-ARS germplasm collection
PI 476016	V	B	17 ± 3	Peru	USDA-ARS germplasm collection
PI 497429	R	L	4 ± 2	Bolivia	USDA-ARS germplasm collection
PI 497598	V	b	67[c]	Ecuador	USDA-ARS germplasm collection
PI 497599	R	L	6 ± 2	Ecuador	USDA-ARS germplasm collection
PI 497669	V	S	80[c]	Peru	USDA-ARS germplasm collection
PI 501273	V	S	90[c]	Peru	USDA-ARS germplasm collection
PI 501983	V	B	17 ± 3	Peru	USDA-ARS germplasm collection
PI 501996	V	B	10 ± 2	Peru	USDA-ARS germplasm collection
PI 502009	R	B	12 ± 2	Peru	USDA-ARS germplasm collection
PI 502039	V	b	50[c]	Peru	USDA-ARS germplasm collection
PI 502154	R	B	12 ± 2	Peru	USDA-ARS germplasm collection
Perry	VR	S	42 ± 5	CV	Isleib et al. (2003)
Southwest Runner	R	S	17 ± 2	CV	Kirby et al. (1998)
Spanco	S	b	24 ± 2	CV	Kirby et al. (1989)
Tamrun 96	R	L	24 ± 3	CV	Smith et al. (1998)
Tamrun 98	R	S	52 ± 5	CV	Simpson et al. (2000)
Tamrun OL02	R	S	62 ± 3	CV	Simpson et al. (2006)
Tamspan 90	S	B	7 ± 1	CV	Smith et al. (1991)

MT, Market Type (R, runner; V, Valencia; S, Spanish; VR, Virginia)

[a] Marker: L = 145 bp band only; B = Both bands present with the 145 bp band being predominant; b = Both bands present with the 100 bp band being predominant; S = 100 bp band only

[b] Origin of genotype refers to (1) line type (i.e. ABL, advanced breeding line; CV, Cultivar) or (2) country of origin if genotype is a plant introduction from the germplasm collection

[c] These genotypes were only tested in replicated plots for 1 year and were omitted from further screening due to their extreme susceptibility to Sclerotinia blight

to remove remaining phenol. DNA was precipitated by the addition of 750 µl isopropanol, spooled on glass hooks and allowed to air dry for 1 h at room temperature. DNA was re-suspended in 100 µl of Tris–EDTA buffer and stored at −20°C until further use.

Analysis, cloning, and sequencing of amplicons

SSR primer pairs reported by Ferguson et al. (2004) were used to examine polymorphism existing among the genotype test set. Amplification using each primer pair was carried out in a PTC-100 thermalcycler (MJ Research, Watertown, MA) under conditions previously optimized for each primer pair (Ferguson et al. 2004). Reaction components: 10 µl (2.5 ng/µl) genomic DNA, 2 µl 10× PCR Buffer, 2 µl 25 mM $MgCl_2$, 1 µl each 10 µM Primers, 2 µl 2 mM dNTP mix, 0.5 µl Hot Start Taq Polymerase (5 U/µl), 1.5 µl H2O. PCR products were visualized by electrophoresis in a 3.5% Metaphor agarose-TAE (Cambrex) gel at 130 V for 6–7 h and subsequent ethidium bromide staining. Bands were identified using Quantity One software (Biorad). Each banding pattern was verified by repeating reactions in triplicate. Total bands amplified were designated as either not polymorphic or polymorphic (data not shown, Chenault and Maas 2005). A total of 16 primer pairs had been used for analysis when a polymorphic band at approximately 275 bp (data not shown) amplified by primer pair pPGPseq2E6R (5′CC TGGGCTGGGGTATTATTT3′) and pPGPseq2E6L (5′TACAGCATTGCCTTCTGGTG3′) was identified to be consistent with Sclerotinia blight resistance and considered a potential marker for that trait.

After identification of the marker associated with Sclerotinia blight resistance, amplicons from 16 different peanut genotypes encompassing all four market types were extracted from excised gel slices using a gel extraction kit from Qiagen, Inc. (Valencia, CA). Amplicons were inserted into the pDrive cloning vector (Qiagen, Valencia, CA) and sequenced with primers SP6 and T7 using an ABI automated sequencer (Oklahoma State University Core Facility, Stillwater, OK). Sequences of all amplicons were compared and a primer termed Marker 3 (5′GCACA CCATGGCTCAGTTATT3′) was designed that is internal to the original left primer (pPGPseq2E6L) but still encompassed the original variable length repeat region. Amplification of the internal fragment at 100–145 bp was performed under conditions described for the original primer pair. Resulting amplicons from 16 genotypes were again extracted from the gel, cloned, and sequenced as previously described. All genotypes tested could be placed into four categories concerning marker presence (Table 1). First, genotypes possessing only the 145 bp band were given a score of L. When genotypes possessed both bands, those with a predominant 145 bp band were scored as B and those with a predominant 100 bp band were scored b. Finally, those genotypes carrying only the 100 bp band were given an S rating. For statistical analysis (Table 2), genotypes receiving scores of L and B were considered a Y (present) while those with b and S ratings were considered an N (absent).

Table 2 Market type by molecular marker comparison of all genotypes tested

Market type	Marker presence	MNSB	SESB
Runner	Y	18.5b	0.85
Runner	N	54.2c	1.88
Spanish	Y	7.1a	0.24
Spanish	N	23.2b	1.41
Valencia	Y	16.7ab	2.84
Valencia	N	71.8c	8.64
Virginia	N	23.5b	2.53

MSNB, Mean percent Sclerotinia blight infection; SESB, standard error for MNSB. Values followed by the same letter are not significantly different using Tukey's comparisons at $P \leq 0.05$

Statistical analysis

Analysis of variances procedures were conducted with the use of PC SAS Version 9 (SAS Institute, Cary, NC) and PROC MIXED. The effects of the presence or absence of marker and market type on the percent Sclerotinia blight were assessed with a two factor factorial arrangement in a randomized block model. The combination of genotype, year, replicate, and investigator were considered random blocking effects. The percent SB response variable was transformed by an arcsine square root transformation in order to alleviate the effects of heterogeneity of variance. The simple effects of marker given market type and the simple effects of market type given marker were evaluated with a SLICE option in an LSMEANS

statement, and if a simple effect was significant at the 0.05 level, pair-wise comparisons of the levels of the factor in question were conducted with a DIFF option and adjusted using Tukey's procedure. Means and standard errors for the combinations of the factors are presented and letters used to represent the observed significant differences.

Results and discussion

The mean percent Sclerotinia blight and standard error values recorded for all genotypes tested and used for marker correlation are shown in Table 1. Of the cultivars tested, Florunner and Okrun averaged the highest disease incidence at 68 and 66%, respectively. Average percent disease among runner type peanuts tested ranged from 2 to 68%, with genotype CS273 having the least disease. Disease among genotypes of the Spanish market-type, which are generally more resistant to Sclerotinia blight due in part to their erect growth habit, ranged from 7 to 22%. Spanish types are underrepresented in the test set compared to runner types due to the limited availability of susceptible lines. Valencia type peanuts, also considered more disease resistant due to growth habit, demonstrated a disease incidence of 8–20% among those genotypes tested for multiple years. Highly susceptible Valencia types PI 497598, PI 497669, and PI 501273 had up to 90% Sclerotinia blight infection and were tested for only 1 year in a resistance screening trial of germplasm accessions, aimed at eliminating extremely susceptible genotypes. Disease incidence among Virginia type peanuts tested ranged from 4 to 42% with the cultivar Perry being most susceptible and ABL N03088FT most resistant. These results were similar to those previously reported for each genotype included in this study, either in published (or non-published) research reports or in cultivar release articles.

Figure 1 illustrates typical marker data collected for each peanut genotype. Using the primers designed to flank the marker; two bands were possible upon amplification: one at approximately 145 bp and one at just over 100 bp. Highly resistant runner genotypes such as PIs 497429, 497599, 274193, and 259796 contained only the band at approximately 145 bp while those with moderate resistance also contained a band at just over 100 bp. In general those runner genotypes considered to have Sclerotinia blight resistance possessed the band at approximately 145 bp at 2–3 times the intensity of the band at 100 bp. Highly susceptible runner genotypes such as Okrun, Florunner, and Tamrun OL02 produced only the band at just over 100 bp upon amplification. The results of resistance marker band scoring for all genotypes are shown in Table 1. Although present in some of the Virginia genotypes tested, the marker band associated with resistance in the runner, Spanish and Valencia market types was not consistent with resistance in any

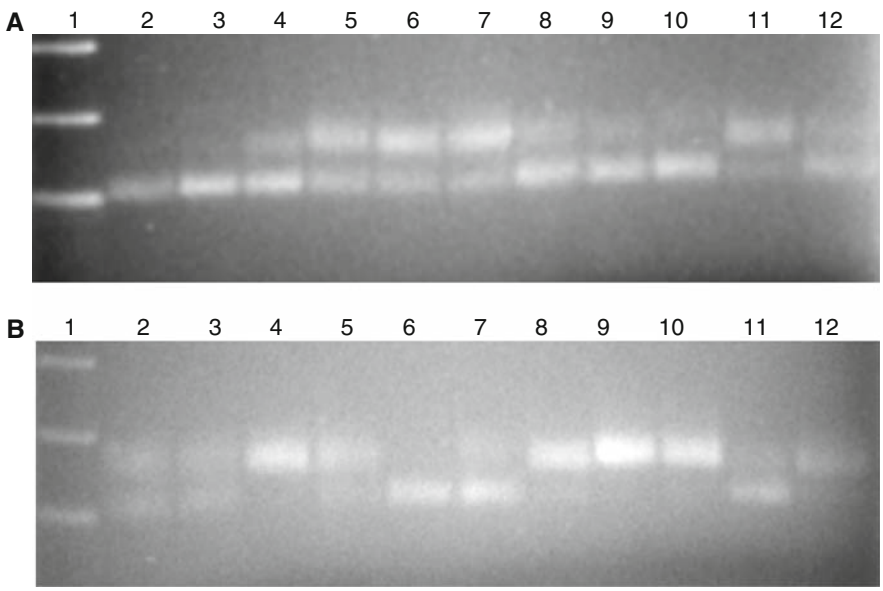

Fig. 1 Example of amplification of peanut DNA using primers pPGPseq2E6R and Marker 3. **a** Lanes 1–12, respectively = 50 bp ladder, Okrun, Flavor Runner 458, Florunner, Georgia Hi-O/L, Georgia Green, Grif 13838, Jupiter, N96076L, Southwest Runner, New Mexico Valencia C, PI 501273. **b** Lanes 1–12, respectively = 50 bp ladder, ARSOK-R1, ARSOK-R2, PI 502009, PI 502154, Tamrun 98, Tamrun OL02, PI 497429, PI 497599, PI 274193, Spanco, Tamspan 90

Virginia genotypes, suggesting a separate source of resistance for that market type and supporting thoughts that Sclerotinia blight resistance is a quantitative trait. The 145 bp band which was present in all resistant genotypes was not present in Southwest Runner which demonstrates excellent resistance to Sclerotinia blight. Although considered a runner peanut, Southwest runner has an extremely erect growth habit and thus an open canopy instead of the dense canopy required for disease progression. Thus, the absence of the 145 bp band in the Southwest Runner genome could be explained by physiological resistance since one of the parents of this cultivar is of Spanish decent (Kirby et al. 1998).

Table 2 shows the correlation of the resistant marker band with Sclerotinia blight resistance and the effect of market type. A graphic representation of SB (%) by marker value for all genotypes (classified by market type) is shown in Fig. 2. For all market types where the 145 bp marker band was present, a correlation was shown between the presence of the band and Sclerotinia blight resistance. The strongest correlation of the marker band and resistance was seen among Spanish genotypes which were significantly different than the runner and Valencia genotypes. These results support any speculation that at least part of the genetic component of Sclerotinia blight resistance may have originated in a Spanish genotype background. When the resistant marker band was not present, the market type effect also becomes apparent, with the Spanish and Virginia types being significantly different from the runner and Valencia types.

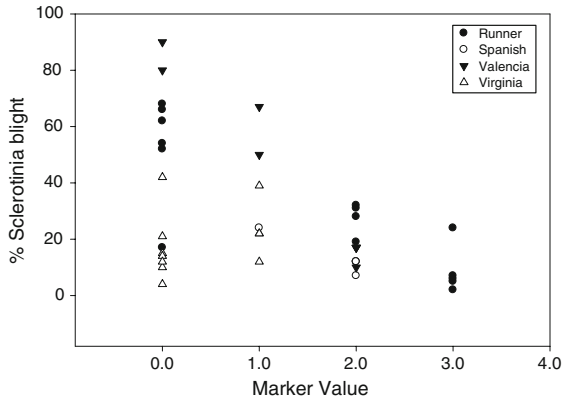

Fig. 2 Scatter plot of SB (%) by marker value according to market type for all genotypes tested. Marker values correspond to those in Table 1 as follows: 0 = S, 1 = b, 2 = B, and 3 = L

These results are consistent with previous studies which have indicated a quantitative behavior under field testing (Wildman et al. 1992; Goldman et al. 1995). There seems to be a distinct dosage effect occurring where the 145 bp band alone provides high levels of resistance, the 100 bp band alone presents considerable susceptability, while a combination of the two confers moderate resistance. For instance, the highly resistant runner genotype PI 497429 possesses only the 145 bp, where as Georgia Hi-O/L has a moderate resistance and carries both the 100 and 145 bp markers. Because peanut cultivars such as those used in this study are typically inbreds produced by single seed decent, the likelihood that the dosage effects are due to heterozygosity is low, which suggests independent loci with an epistatic effect.

The possibility of marker correlation with resistance being due to kinship of the peanut genotypes examined in this study is minimal. The deliberate inclusion of plant introductions of the USDA-ARS peanut germplasm collection which were not breeding lines or cultivars, but were introductions collected at different times from various countries around the world served as an internal control against kinship correlations. Furthermore, extensive pedigree examination of all cultivars or ABLS for each market type revealed no obvious kinship across genotypes possessing a similar banding pattern. For example, the once widely grown cultivar Florunner which is extremely susceptible to Sclerotinia blight and contains only the 100 bp marker band, is a common ancestor, although not necessarily immediate, to many of the runner cultivars or ABLs (resistant and susceptible) included in this study. However, there is no common PI, cultivar or breeding line that was crossed with Florunner to produce resistant progeny lines. The same can be said of the pedigrees of the Spanish and Valencia genotypes included in this study as plant introductions from foreign countries were also included in the test set of those market types.

Figure 3 illustrates partial sequence data obtained from the marker fragments of 16 genotypes. The sequence shown surrounds the SSR area which was the only region that was not well conserved. The sequence data obtained supports the size differences in amplified bands from the different genotypes. Resistant genotypes, shown in red, have a longer repeat region (19–34 CT repeats) while susceptible

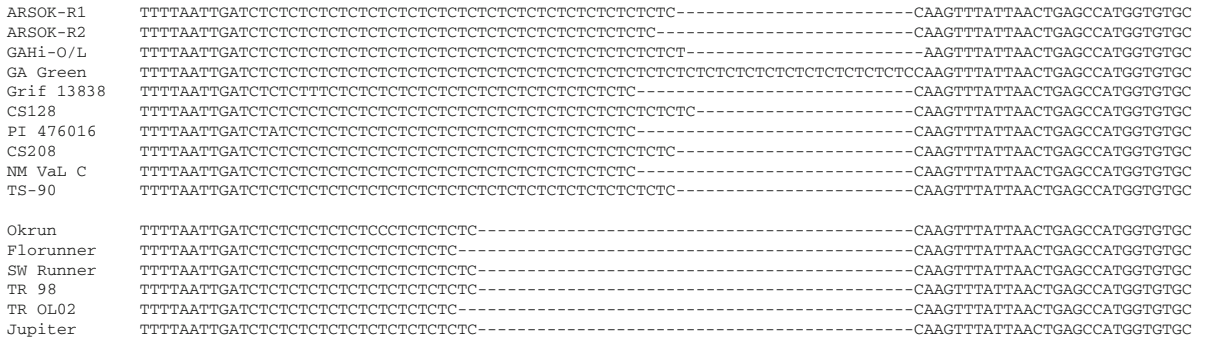

Fig. 3 Alignment of partial sequence data obtained from cloned fragments of peanut genomic DNA amplified by primers pPGPseq2E6R and Marker 3

genotypes contain less (11–12 CT repeats). Although in general the length of the repeat is consistent with either resistance or susceptibility, there is no apparent correlation with repeat length and degree of resistance (i.e. the cultivar Georgia Green has the longest repeat but not the highest level of resistance). BLAST searches were conducted on several data bases including the NCBI-Entrez Nucleotide Database, the Legume Information System (LIS) and TIGR Gene Indices (nucleic acid) and did not suggest a matching identifiable motif or gene.

This is the first report of a molecular marker associated with resistance to Sclerotinia blight in peanut. Because this marker amplifies fragments of different sizes from susceptible and resistant plants, much like the marker recently developed for nematode resistance in peanut (Chu et al. 2007), the possibility of false diagnosis due to amplification failure can be avoided. Work is currently underway to place this marker on the genomic map of peanut, and to identify possible QTL(s) associated with the trait. The marker will have great utility in screening not only germplasm collections but also segregating populations. The use of marker assisted selection in screening segregating populations for resistance will allow for earlier generation testing of breeding lines and effectively reduce the number of years of greenhouse and/or field trial testing needed for cultivar release. Peanut breeders will be able to rapidly identify peanut germplasm and breeding lines for the rapid selection of elite breeding material with the potential for high levels of Sclerotinia blight resistance.

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